

(From B.M.J., Nov. 14th, 1903.)

NOTE ON THE BODIES RECENTLY DESCRIBED BY LEISHMAN AND DONOVAN.

BY MAJOR R. ROSS, F.R.S., F.R.C.S., C.B.,

Professor of Tropical Medicine, University of Liverpool.

(From the Johnston Tropical Laboratory, Liverpool.)

IN May, 1903, Major Leishman, R.A.M.C., described¹ certain small oval bodies occurring in smear preparations made *post mortem* from the spleen of a soldier suffering from low fever, chronic dysentery, and cachexia. The observation was made in London at the Military Hospital, and the patient had contracted his illness at Dum-Dum, near Calcutta. Leishman gave a very accurate description of these bodies. He said that they existed in large numbers among the spleen cells and red corpuscles; that they were round or oval, and $2\ \mu$ to $3\ \mu$ in diameter; that on staining with Romanowsky's method they were found to contain two masses of chromatin—a large circular mass or ring, and another smaller mass, “usually in the form of a short rod set perpendicularly or at a tangent to the circumference of the larger mass.” The outline of the bodies containing these two masses of chromatin was faintly visible with this stain. The bodies were usually isolated, but occasionally aggregated into “clumps composed of twenty to fifty members.” Regarding their nature, he thought it probable that they were the residue of trypanosomes of which the vibratile membrane had disappeared after death, leaving behind the nucleus and micronucleus shrunk together in a small mass, and quotes in support of this view the occurrence of similar *post-mortem* changes in the trypanosomes of dead rats.

In July Captain Donovan, I.M.S., stated² that he had already found the same bodies on April 9th, 23rd, and 24th in *post-mortem* smears from the spleens of three consecutive cases said to have died from chronic malaria in Madras. At first he thought that the bodies represented the long-sought-for resting stage of the malaria parasite, but, after finding them in two other cases, began to think that they were *post-mortem* degenerations of spleen cells. On reading Leishman's paper he recognized their similarity to the bodies described by that observer; and next was able to recover them in blood taken *intra vitam* from the spleen of a boy suffering from irregular pyrexia (June 17th). He remarks that this case excludes the possibility of the bodies being due to *post-mortem* changes, and adds that nothing resembling trypanosomata could be found in the boy's blood.



Captain Donovan was good enough to send me three preparations well stained by the Romanowsky method—namely, one preparation from his case of April 9th (*post mortem*), one from his case of June 17th (*intra vitam*), and a third dated July 4th from the splenic puncture (*intra vitam*) of yet another case. I have examined these carefully, and as there has already been considerable delay over them I think that the following brief notes should be recorded at once as a verification of the previous papers:

1. All these preparations contain bodies agreeing exactly with Leishman's description, and he himself admits the similarity after having been so kind as to examine the specimens.

2. In Donovan's specimen of April 9th (*post mortem*) the bodies number about 30 in a field and measure about 2.5μ . They are disconnected from each other, but often lie fairly close together in groups of 10 or more. In the specimen of June 17th (*intra vitam*) they number only about 1 in 12 fields, but are slightly larger—up to 3.25μ . In that of July 4th they are also large, and number about 1 in every field or so; while groups of from 2 to 12 of them can often be found embedded in what seems to be some kind of matrix which stains a faint bluish-grey colour, and which has an oval contour measuring up to 8μ in the long diameter. Some of the bodies appear even to be intracorpuseular, but this may be due to accidental superposition.

3. Both large and small nuclear masses are almost invariably present, and both are always stained a deep vivid crimson. The smaller one is very often rod-like and pointed at the large one, just as Leishman describes, and stains somewhat deeper than it. The bioplasm is generally colourless, but its contour is reddish in the specimens made *intra vitam*.

4. In none of the specimens can I find either entire trypanosomes or the remains of dead ones, such as the characteristic flagella.

It is to be hoped that Major Leishman and Captain Donovan will be able shortly to elucidate the nature of these very interesting objects. Their size and shape and the arrangement of their chromatin are so constant and characteristic that it is scarcely possible to doubt that they are parasites of some kind, probably protozoa. For my own part, I find some difficulty in adopting Leishman's view that they represent involution stages of trypanosomes in the *post-mortem* spleen. He describes accurately such forms in the rat; but there, it should be carefully noted, the stained flagella are always present (with a successful Romanowsky technique) in addition to the macronucleus and micronucleus, and are, indeed, the last of the elements to refuse colouration. If, then, these bodies are nothing but disintegrating trypanosomes, we should certainly find a few of the flagella among them. Then, again, neither the large nor the small nucleus in the preparations seems to me to recall those of trypanosomes; and they are, moreover, approximated in a much more regular manner than, I think, we observe in dead trypanosomes.

Lastly, as Donovan observes, his two preparations made *intra vitam* exclude *post-mortem* changes altogether, and should, therefore, contain some unaltered trypanosomes. Prima facie, then, I am strongly inclined to think that we

have to do with some quite novel organism. As it has already been found in eight cases of fever and cachexia, it promises to be a common and important one. The charts of two of Donovan's cases sent by him to me certainly recall the chronic pyrexia with enlarged spleen so frequently observed in India, and are, indeed, not a little suggestive of kala-azar.

REFERENCES.

¹ Leishman, On the Possibility of the Occurrence of Trypanosomiasis in India, BRITISH MEDICAL JOURNAL, May 30th, 1893, p. 1252. ² Donovan, *ibid.*, July 11th, 1903, p. 79.

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FURTHER NOTES ON LEISHMAN'S BODIES.

By MAJOR RONALD ROSS, F.R.S., F.R.C.S., C.B.,
Professor of Tropical Medicine, University of Liverpool.

I HOPE that the interest which must always attach to the discovery of new parasites of man will suffice to excuse me for adding yet another paper on this subject to those already contributed by Leishman,¹ Donovan,² Laveran,³ and myself.⁴ I have continued making a very close study of the three preparations so kindly lent to me by Donovan and referred to in my last paper, and now send, at the suggestion

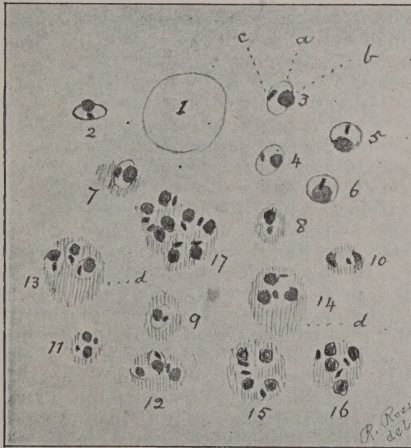


Fig. 1.—*Leishmania donovani*, Laveran; 1, red corpuscle; 2-6, free forms; 7-17, embedded forms; a, contour line; b, macronucleus; c, micronucleus; d, matrix.

of the editor, some pen and ink drawings, and some additional remarks. It will be understood that the deep red of the Romanowsky-stained chromatin of the bodies is represented by black in the drawings, and that Fig. 1 denotes a red corpuscle introduced for comparison. Water-colour drawings by Donovan and by myself will shortly appear in the Thompson Yates Reports.

It seems to me very important to note that in the preparations made *intra vitam* Leishman's bodies appear under two distinct conditions, namely, (a) free, and (b) embedded, to the number of one up to twelve, in a matrix. Figs. 2 to 6 illustrate the free forms. In the majority the contour is elliptic, but it is occasionally nearly circular. It is necessary to recognize that the contour line is a strong one, suggestive of a well-marked cell wall (that is, in the *intra-vitam* specimens, which are, of course, the most suitable for study). The two chromatin masses are generally situated at the extremities of the minor diameter of the elliptic cell (Figs. 2 to 5). The larger mass often seems to bulge beyond the cell wall (Figs. 2 to 4). I think that both masses are always present, but that the smaller one may be occasionally hidden by the larger. In my specimens the protoplasm of the cell is a faint red (without tinge of blue), and becomes fainter from the contour inwards, so that there is the characteristic clear area round the macronucleus. In short, all the free forms have a most definite size and structure.

Much interest, however, attaches to those bodies which are embedded in a matrix (Figs. 7 to 17). These are found only in the preparations made *intra vitam*, and are much less numerous than the free forms. Moreover the contour of the little cell is generally much less distinct in the embedded organisms than in the free ones; although the chromatin is stained just as deeply and the two chromatin masses bear just the same relations to each other as regards position and distance. What I call (until a more definite term can be employed) the matrix is always stained very faintly in these specimens—much more faintly than the red or white corpuscles. The tint is violet, or more rarely mauve. The structure appears cloudy, or perhaps granular, or even stromatic; but is too delicate for expression in the drawings. The form is generally a more or less regular oval (Figs. 8 to 16); but sometimes the mass appears to be shapeless or torn (Figs. 7 to 17). The outline is sharp; but it is important to note that there is never any contour line suggestive of a cell-wall, as with the Leishman bodies themselves. There is never, also, any suggestion of the haemoglobin of the red corpuscles, or the nucleus of the white corpuscles, to be seen in this matrix. Its size varies in my specimens from about 3μ to 8μ in the long diameter; and there is a rough, but only a rough, correspondence between the size of the matrix and the number of Leishman bodies it contains. Sometimes, as in Fig. 13, a considerable part of the matrix is empty; and sometimes, a rather large matrix holds only one of the bodies. Fig. 17 shows eight bodies in one matrix: but I have seen as many as twelve. Occasionally we find clusters of the bodies touching each other, without lying in any visible matrix; but in such cases their oval contours are well marked. One or more of the micronuclei are sometimes not to be seen. I should add that Donovan clearly shows, in his letter and drawings sent to me, that he has well observed all these forms.

In his recent paper⁵ Leishmann still inclines to the view that these bodies are "altered trypanosomes." In that case it is singular that in two whole specimens made during life and containing large numbers of them, we should not find a single unaltered trypanosome, or even the flagellum of one. I may be wrong, but I can see little in these objects to recall the involution forms of trypanosomes.

Since my previous paper¹ was dispatched (October 23rd, see BRITISH MEDICAL JOURNAL, p. 1359) Dr. Laveran, to whom also Captain Donovan has sent specimens, read an interesting paper on the subject.³ He admits that the bodies are parasites; but thinks they are neither trypanosomes nor haematozoa (malaria group), and judges that they belong to the Texas cattle-fever group, *Piroplasma*. According to him the bodies are often contained in the red corpuscles, and are sometimes piriform. There is little evidence of this in the preparations sent to me; I have examined some thousands of the bodies, and have seen only two or three lying in contact with the red corpuscles (which are well preserved in two of the specimens). I am therefore inclined to attribute the contact only to accidental superposition. Nor have I observed any distinctly piriform bodies in my preparations. The matrix of the embedded forms does not present to me any of the characters of a red corpuscle altered by parasitic infection.

On the whole then, I think these bodies belong to a new genus of *Sporozoa*; and it seems to me, so far as I can judge from my specimens, that the individual bodies are spores produced in the matrices, which would appear to be relics of the parent organism.

Laveran has given the name *Piroplasma donovani* to these organisms; and the specific name must therefore be permanently adopted. But if, as I suppose, they are found to belong to a new genus, it would be only fair to give the name *Leishmania* to that genus. In that event the full name would be *Leishmania donovani*, Laveran.

I must present my profound apologies to Major Leishman for having overlooked the fact, clearly indicated in his first paper, that he had found these bodies as long ago as 1900.

REFERENCES.

- ¹ Leishman, BRITISH MEDICAL JOURNAL, 1903, vol. i, p. 1252, and p. 1376.
² Donovan, *ibid.*, vol. ii, p. 79. ³ Laveran, *Bull. Acad. de Méd.*, November 3rd, 1903. ⁴ Ross, BRITISH MEDICAL JOURNAL, 1903, vol. ii, p. 1261.

Editorial Note, British Medical Journal, Nov. 21st, 1903, p. 1359.

IN connexion with the communication from M. Laveran to the Académie de Médecine of Paris on November 3rd, in which he described a parasite on the strength of specimens sent to him by Captain Donovan, we desire to state that the paper by Major Ronald Ross dealing with this subject, published in the BRITISH MEDICAL JOURNAL of November 14th, p. 1261, was received by us on October 24th. M. Laveran believes that the parasite is the piroplasma, and proposes to give it the name *P. donovani*. Another piroplasma, *P. bigeminum*, is the cause of Texas fever of cattle, and other species occur in the sheep, horse, and dog.

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